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Invention: MEDICAMENT

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SPECIFICATION

Medicament

The present invention concerns treatment, prevention and diagnosis of infection due to *Chlamydia pneumoniae* and in particular to the prevention and treatment of atherosclerosis, including coronary atherosclerosis, caused by same.

C. pneumoniae is associated with atherosclerosis but no definitive link between the two has yet been established (Hammerschlag, M.R., 1998, Eur. J. Clin. Microbiol. Infect. Dis., 17: 305-308). Friedank, H.M. *et al.* (1993, Eur. J. Clin. Microbiol. Infect. Dis., 12(12): 947-951) identify a 54 kDa *C. pneumoniae* antigen which was recognised by 93% of sera positive for *C. pneumoniae*, the antigen appearing to be located on the surface of elementary bodies. Wiedman, A.A.M. *et al.* (1997, Clin. Diagn. Labs. Immunol., 4(6):700-704) showed the infectivity of *C. pneumoniae* elementary bodies to be slightly reduced by the use of antibody specific against a 54 kDa *C. pneumoniae* protein.

Despite investigating it, other researchers have not confirmed the immunogenicity of the *C. pneumoniae* 54 kDa band (see for example Kutlin, A. and Roblin, P.M., 1998, J. Infect. Dis., 177: 720-724; Campbell, L.A. *et al.*, 1990, J. Clin. Microbiol., 28(6): 1261-1264; Campbell, L.A. *et al.*, 1990. Infection and Immunity, 58(1): 93-97; Puolakkainen, M. *et al.*, 1993, J. Clin. Microbiol., 31(8): 2212-2214; hkima, Y. *et al.*, 1994, J. Clin. Microbiol., 32(3): 583-588; Maass, M. and Gieffers, J., 1997, J. Infection, 35: 171-176; Gonen, R. *et al.*, 1993, APMIS, 101:719-726).

The present inventor has now succeeded in isolating, purifying and identifying a *C. pneumoniae* protein which (together with inhibitors of same, such as

antibodies) is protective and therapeutic against *C. pneumoniae* infection. The therapeutic role of the protein has previously neither been suggested nor disclosed.

According to the present invention there is provided a *C. pneumoniae* protein having the amino acid sequence of SEQ ID NO: 2, for use in a method of treatment or diagnosis of the human or animal body. The amino acid sequence has been confirmed by N-terminal amino-acid sequencing (see "Experimental" below) and the protein has a theoretical molecular weight of 50.8 kDa, although post-translational modifications such as glycosylation may of course affect its apparent molecular weight as determined by e.g. SDS-PAGE. Experiments (below) have shown it to have an apparent molecular weight of 51 kDa on SDS-PAGE gels.

As can be seen from the plethora of publications above, although some identify immunogenic bands at molecular weights of 50-54 kDa, no specific therapeutically effective proteins have been identified.

Experiments (below) have allowed the present inventor to isolate and purify the protein of the present invention and identify the gene sequence coding for the protein. This has allowed the determination of the protein amino acid sequence (above). The nucleotide sequence coding for same forms another part of the present invention. Thus according to the present invention there is also provided a nucleotide sequence coding for a protein according to the present invention, for use in a method of treatment or diagnosis of the human or animal body. Such a nucleotide sequence may have the sequence of SEQ ID NO: 1. Modified nucleotide sequences having codons encoding the same amino acid sequence will be readily apparent to one skilled in the art.

The nucleotide sequence of the present invention and the amino acid sequence it encodes are already known from the Chlamydia Genome Project

(*C. pneumoniae* CWL029/CPn0809), as is an apparent *C. trachomatis* homologue (CT578). However, therapeutic and diagnostic uses for same have not been previously suggested.

The invention also extends to encompass forms of the protein which have been insubstantially modified (i.e. which have been partially modified), particularly forms of the protein which display the same immunogenic properties as the protein itself.

By "partial modification" and "partially modified" is meant, with reference to amino acid sequences, a partially modified form of the molecule which retains substantially the properties of the molecule from which it is derived, although it may of course have additional functionality. Partial modification may, for example, be by way of addition, deletion or substitution of amino acid residues. Substitutions may be conserved substitutions. Hence the partially modified molecule may be a homologue of the molecules from which it was derived. It may, for example, have at least 70% homology with the molecule from which it was derived. It may for example have at least 80, 90 or 95% homology with the molecule from which it was derived. An example of a homologue is an allelic mutant.

Also provided according to the present invention is the use of a protein, immunogenic fragment thereof or nucleic acid sequence encoding same according to the present invention in the manufacture of a medicament for the treatment of infection due to *C. pneumoniae*.

Immunogenic fragments of the protein include any fragment of the protein which elicits an immune response, and includes epitopes. Analogues (mimotopes) of epitopes may be readily created, the mimotopes having different sequences but displaying the same epitope and thus the term "immunogenic fragments" also

encompasses immunogenic analogues of the fragments e.g. mimotopes. Epitopes may be readily determined and mimotopes readily designed (Geysen, H.M. *et al.*, 1987, Journal of Immunological Methods, 102: 259-274; Geysen, H.M. *et al.*,1988, J. Mol. Recognit., 1(1):32-41; Jung, G. and Beck-Sickinger, A.G., 1992, Angew. Chem. Int. Ed. Eng., 31: 367-486). Such an immunogenic fragment carrying epitopes may also be described as being a peptide having the amino acid sequence of the immunogenic fragment and which carries an epitope.

The present inventor has succeeded in isolating a number of epitopes (immunogenic fragments) of the protein of the present invention. Thus according to the present invention there is also provided an epitope having the amino acid sequence of any one of SEQ ID NOs: 4-14. In particular, SEQ ID NOs: 5-7 provide an overlapping set of highly immunogenic peptides - as can be seen from the experimental data (below) SEQ ID NO: 5 provides for especially good results. Similarly, excellent results are also obtained from SEQ ID NO: 8.

The protein, immunogenic fragments thereof and nucleic acid sequences encoding same may be used in therapy, both prophylactically (e.g. as immunostimulants such as vaccines) and for treatment of infection due to *C. pneumoniae*. For example a nucleotide sequence encoding the protein or immunogenic fragment thereof may be used in the manufacture of a DNA vaccine (Montgomery, D.L. *et al.*, 1997, Pharmacol. Ther., 74(2): 195-205; Donnelly, J.J. *et al.*, 1997, Annu. Rev. Immunol., 15: 617-648; Manickan, E. *et al.*, 1997, Crit. Rev. Immunol., 17(2): 139-154).

Binding agents and inhibitors (such as antibodies or other neutralising agents) specific against the protein and immunogenic fragments thereof may also be used both diagnostically and therapeutically. Binding agents have a target to which they are specific, and in the case of a binding agent being an antibody, the target is an antigen.

An example of a therapeutic medicament is antibody specific against the protein of the present invention, and this may be employed in immunotherapy, for example passive immunotherapy. Antibodies, their manufacture and use are well known (Harlow, E. and Lane, D., "Using Antibodies - A Laboratory Manual", Cold Spring Harbor Laboratory Press, New York, 1998) and so antibodies and antigen binding fragments thereof will be readily apparent to one skilled in the art, and reference herein to antibodies is also reference to antigen binding fragments unless stated otherwise. Other inhibitors such as ribozymes, antisense oligonucleotides and DNA vaccines will be readily apparent to one skilled in the art (Fries, P.C., 1999, "DNA Vaccines", New England Journal of medicine, 341: 1623-1624; Leitner, W.W. *et al.*, 1999, "DNA and RNA based vaccines: principles, progress and prospects", Vaccine, 18: 765-777; Muotri, A.R. et al., 1999, "Ribozymes and the anti-gene therapy: how a catalytic RNA can be used to inhibit gene function", Gene, 237: 303-310; Rossi, J.J., 1999, "Ribozymes, genomics and therapeutics", Chemistry & Biology, 6: R33-R37; James, H.A., 1999, "The potential application of ribozymes for the treatment of haematological disorders", Journal of Leukocyte Biolofy, 66: 361-368)

Thus the present invention also provides the use of a inhibitor specific to the protein of the present invention in the manufacture of a medicament for the treatment of infection due to *C.pneumoniae.*

Also provided according to the present invention is a method of manufacture of a medicament for the treatment of infection due to *C. pneumoniae*, characterised in the use of a protein, immunogenic fragment or inhibitor according to the present invention.

Also provided according to the present invention is a method of treatment of infection due to *C. pneumoniae*(e.g. of a patient in need of same), comprising the step

of administering to a patient a medicament comprising a protein, immunogenic fragment or inhibitor according to the present invention. The exact dose of medicament administered to a patient may be readily determined using simple dose-response assays. Medicaments may additionally comprise a pharmaceutically acceptable carrier, diluent or excipient (Remington's Pharmaceutical Sciences and US Pharmacopeia, 1984, Mack Publishing Company, Easton, PA, USA)

It has not been previously suggested that the protein of the present invention (or immunogenic fragments of same) is diagnostic for infection due to *C. pneumonia*. Binding agents specific to the protein of the present invention (for example antibodies) may also be used diagnostically, for example in an ELISA-type test. Thus also provided according to the present invention is the use of a protein, immunogenic fragment or binding agent according to the present invention in the manufacture of a diagnostic test for *C. pneumoniae*.

Also provided is a diagnostic test method for infection due to *C. pneumoniae* comprising the steps of:

I) reacting an antibody specific against the protein of the present invention with serum from a patient;

ii) detecting an antibody-antigen binding reaction; and

iii) correlating the detection of an antibody-antigen binding reaction with the presence of the protein.

Such test methods may also be performed using other binding agents specific to the protein of the present invention.

Also provided is a kit of parts for performing such a test, characterised in that it comprises antibody specific against the protein of the present invention.

The invention will be further apparent from the following description, with reference to the several figures of the accompanying drawings, which show, by way of example only, uses of the proteins of the present invention.

EXPERIMENTAL

The experiments below detail the identification of a number of peptides and antisera against same which are useful in the therapy and diagnosis of infections due to *Chlamydia pneumoniae*. Starting with sera from infected patients, blotting against clinical isolates of *Chlamydia pneumoniae* showed the presence of an immunodominant antigen with an apparent molecular weight of 51 kDa, the antigen being stable to and released by octylglucoside treatment. N-terminal amino acid sequencing of the protein of the 51 kDa band allowed sequence database probing, in turn identifying a *C. pneumoniae* protein and a *C. trachomatis* homologue. Epitope mapping allowed the identification of antigenic peptides, which together with antibody against them were tested for their therapeutic and diagnostic efficacy.

Western Blotting - Using the Novex nuPAGE Electrophoresis System.

1. SDS PAGE

Preparation of Sample:

1. 100 µl of Novex SDS Sample loading buffer was added to 400 µl of a preparation of a *Chlamydia pneumoniae* clinical isolate and the mixture placed into a boiling waterbath for 10 minutes.

2. 10 µl of the mixture was loaded into each well of a Novex 4-12% Bis-Tris NuPage gel (1.0 mm, 12 well). In addition, 4 µl of Novex Multimark molecular weight standards were added to a single well on each gel.

3. Electrophoresis was performed using 1x Novex MOPS electrophoresis buffer at 200v for 40 minutes.

Western Transfer Protocol:

1. The blotting apparatus and the gel membrane "sandwiches" were assembled according to the protocol described in the Novex instruction booklet provided with the gels.

2. Blotting was performed using 1x Novex Transfer buffer containing 20% methanol. Transfer was carried out at 30v (constant) for 1 hour.

3. Following transfer, the membranes were removed from the apparatus and left to "Block" overnight in 3% Bovine Serum Albumin (BSA) at 4 °C.

Probing With Patient's Serum:

1. The membranes were cut into strips and placed into the wells of incubation trays. Patients' serum was diluted 1 in 20 in 3% BSA and 2 ml added to each strip. (2 strips per patient).

2. The membranes were incubated at room temperature for 2 hours with agitation.

3. The strips were washed 5 times over 30 minutes with 0.85% NaCl/0.01% Tween 20.

4. 2 ml of goat anti-human IgM or IgG alkaline phosphatase conjugated anti-immunoglobulin diluted 1 in 4000 in 3% BSA were added to each strip. The strips were incubated for a further hour at room temperature with agitation.

5. The membranes were washed a further 5 times as previously described.

6. Antibody-antigen interaction was visualised by the addition of NBT/BCIP (50 mg/ml) in pH 9.5 phosphate buffer.

7. The reaction was allowed to proceed until the bands had reached the required intensity.

Sera

Group A: Children with respiratory tract infection and no evidence of *Chlamydia pneumoniae* as shown by negative microimmunofluorescence (less than 1 in 64) test (n=19).

Group B: Children with respiratory tract infection and a microimmunofluorescence titre greater than 1 in 512 (n=18).

Group C: Patients undergoing cardiac surgery for advanced coronary disease (n=32). Ten of these had antibody on immunoblot.

Group D: Adults with respiratory tract infection and a chlamydia complement fixation test greater than 1 in 40 (n=27) using LGV 2 as an antigen.

Group E: Adults with pelvic inflammatory disease due to *Chlamydia trachomatis* (n=21).

Group F: Sera (n=11) which were positive for the 60/62 kDa doublet and band at 51 kDa were retested on antigen prepared from *Chlamydia pneumoniae* where the purified elementary bodies were incubated with 1% octylglucoside at 37 °C for 30 minutes rather than in SDS.

Results:

Results of the sera blotting experiments are shown in Table 1. It should be noted that sera blotting determines the presence in patients of antibodies specific against a given antigen, and so when a patient has previously been infected by a pathogen and developed an immune response against an antigen, that immune response may still be detectable at a later date when the patient is no longer infected. Hence background results must be interpreted in light of the general infection of a population by the pathogen. For example, the general population has an infection rate by adulthood of approximately 10% for *C. pneumoniae*, thus a background rate of detection of *C. pneumoniae* antigens of up to 10% should be expected.

Conclusions:

The sera from Group A children did not recognise *C.pneumoniae* on immunoblot. The Group B sera from children with evidence of *C.pneumoniae* infection recognised a range of antigens with apparent molecular weights ranging from 30 to 180 kDa. IgM for an antigen complex at 60/62 kDa which occurred as a doublet was immunodominant as well as an antigen at 51 kDa. For IgG the antibody was most pronounced for the antigen at 51 kDa. In the cardiac patients, 23 produced antibody and this was for IgM against the bands at 67, 60/62 and 51 kDa. For IgG this was the band at 51 kDa. For Group D IgM was most pronounced for the 60/62 kDa doublet and IgG for the band at 180 kDa and the doublet at 60/62 kDa. This group of sera contains those with infection most likely due to *Chlamydia psittaci*. The sera from Group E patients infected with *Chlamydia trachomatis* did not cross-react.

Group F Sera

On re-blotting with those sera previously positive for the 60/62 kDa doublet and 51 kDa, the doublet disappeared whilst the band at 51 kDa remained. This showed that the band at 51 kDa was stable to and released by octylglucoside treatment.

Solubility in Octylglucoside

Using samples from Group F patients, separation of antigens from elementary bodies using 1-D gel electrophoresis and SDS gave a different staining pattern compared to using 1-D gel electrophoresis and octylglucoside. The 51 kDa band was still visible after octylglucoside. The pair of antigenic bands at 60/62 kDa was not visible in octylglucoside. Therefore a distinguishing character of the 51 kDa antigen of the present invention is its solubility in octylglucoside.

N-Terminal Amino Acid Sequencing

N-Terminal amino-acid sequencing was performed upon the 51 kDa band. The resulting sequence was then used to query the Chlamydia Genome Project database which identified the protein of SEQ ID NO: 2 and a *C. trachomatis* homologue.

Epitope Mapping

A series of overlapping peptides of 15 amino acids covering the derived amino acid sequence of the protein were synthesised on polyethylene pins with reagents from an epitope scanning kit (Cambridge Research Biochemicals, Cambridge, UK) as described previously by Geysen *et al.* (1987, Journal of Immunological Methods, 102: 259-274). Peptide 1 consisted of residues 1 to 15, peptide 2 consisted of residues 2 to 16 etc. The reactivity of each peptide with patient sera (diluted 1:200) was determined for IgG by ELISA. Data were expressed as A405 after 30 minutes of incubation.

Sera from patients as follows:

Group 1: Children with respiratory tract infection and no evidence of *Chlamydia pneumoniae* as shown by negative immunoblot and microimmunofluorescence (less than 1 in 64) (n = 3).

Group 2: Children with respiratory tract infection, positive immunoblot and microimmunofluorescence test greater than 1 in 512 (n = 6).

Group 3: Patients undergoing cardiac surgery for advanced coronary disease and antibody on immunoblot (n =2).

Group 4: Patients presenting with history of chest pain, negative troponin (<0.2), negative immunoblot (n = 3).

Group 5: Patients presenting with early coronary, positive troponin (>0.2) and antibody on immunoblot (n =8).

Results

Epitope mapping

Epitope mapping defined eleven areas where children with acute chlamydial infection produced wells with a mean optical density (OD) greater than 1. In the case of epitopes having SEQ ID NOs: 4, 5, 6, 7, 8, 10, 12 and 14 the mean OD was at least 2 standard deviations above that of Group 1 (children with no evidence of *C.pneumoniae* infections). This applied also to Groups 3, 4 and 5 with the exception of SEQ ID NO: 5 which was positive in Groups 4 and 5.

Peptide 1(SEQ ID NO: 15) representing epitope having the sequence of (i.e. which is carried by the peptides having the sequence of) SEQ ID NO: 8 and peptide 2 (SEQ ID NO: 16) representing the carboxy end of SEQ ID NO: 4, the epitope having the sequence of SEQ ID NO: 5 and the amino end of SEQ ID NO: 6 were synthesised.

Preparation of rabbit polyclonal serum

New Zealand white rabbits were pre-bled and then immunised subcutaneously with either peptide 1 or peptide 2 (0.1 ml of 1 mg/ml) conjugated to KLH suspended in either Freund's adjuvant (injection at day 0) or Freund's incomplete adjuvant on days 14, 42, and 70). Serum was obtained for indirect ELISA at the terminal bleed-out.

Indirect ELISA

By a simple adsorption of each peptide to a microtitre plate the following procedure was performed The peptide was dissolved in 2 ml of 0.01 M phosphate buffer saline (PBS), pH 7.2 and diluted to a concentration of 10 μg/ml (1/100) in the same buffer.

1. 150 μl aliquots of peptide (10 μg/ml in 0.01 M PBS) were pipetted into the wells of a Falcon 3912 microassay plate and were incubated overnight at 4 °C.
2. The unbound peptide was removed by washing four times (4 x 10 minutes) with 0.05% Tween 20 in 0.01 M PBS (pH 7.2).
3. The plates were blocked with 2% skimmed milk-10% FCS in 0.01 M PBS for 1 hour at 37 °C.
4. The plates were washed four times (4 x 10 minutes) with 0. 05% Tween 20 in 0.01 M PBS and the serum under investigation was added (1/100 dilution in blocking solution) into the wells of micro assay plate (three wells used for each serum) and incubated for 2 hours at 37 °C.
5. The plates were washed four times (4 x 10 minutes) with 0.05% Tween 20 in 0.01 M PBS and secondary antibody, anti-rabbit IgG peroxidase conjugate (1/1000 dilution in blocking solution) was added and incubation proceeded for 1 hour at 37 °C.
6. The plates were washed four times (4 x 10 minutes) with 0.05% Tween 20 in 0.01 M PBS, followed by a further washing with 0.01 M PBS. The plate was then incubated for 45 minutes at room temperature with agitation in 0.5

mg/ml of freshly prepared 2,2 Azino-bis [3-ethylbenz-thiazoline-6-sulfonic acid] diammonium (ABTS tablets) in pH 4.0 citrate buffer with 0.01% (w/v) hydrogen peroxide.

7. Optical density (OD) measurements were made with an ELISA plate reader (Titertek Miltiscan) at a wavelength of 405 nm.

8. The average readings for each three wells for each serum was determined.

Results

The results shown in Table 3 demonstrate seroconversion to each individual peptide.

Expression of the amino-end of the protein

The sequence was codon optimised (Genosys, California) for *E.coli* and a BamHI and Not1 site added to opposite ends. The optimised sequence and PET 29 vector (Novagen, Wisconsin) were restriction digested using BamHI and Not1 and transformed by heat shock into *E.coli* strain BL21 (Invitrogen, Carlsbad, California). The expressed amino acids were from amino acids 1-292 and included the epitopes represented by peptides 1 and 2. This construct included an S-tag and Thrombin cleavage site at the amino end and histidine tag at the carboxy end (SEQ ID NO: 3).

Purification

The transformants were expressed as follows. Briefly, 5 ml of an overnight culture was used to inoculate 500 ml LB (50 µg/ml kanamycin, 34 µg/ml chloramphenicol) which was grown for 2 hours at 37 °C to an OD 600 of 0.5, then induced for 3 hours with 0.1 mM IPTG (Sigma, Poole Dorset). The cells were pelleted and disrupted by crushing at -20 °C in an XPRESS. The buffer (50 mm NaH_2PO_4, 0.5 M NaCl, 10 mm imidazole) and the cell debris pelleted down. The supernatant was filter sterilised and put on a Ni-NTA agarose slurry affinity column (Qiagen) in order to capture the His-tagged recombinant protein. The column was washed 3 times with 4 ml of washing buffer and

the protein eluted maximally with 150 mM imidazole. The protein gave a single band on a 10% acrylamide gel stained with Coomassie Brilliant Blue with an apparent molecular weight of 37 kDa. On Western blot counterstaining with the anti-His mouse alkaline phosphate conjugate (1:2,500) (Sigma, Dorset, Poole) this produced a single band at 37 kDa and a breakdown product at 35 kDa. The protein concentration of the elute was measured and standardised to 10 mg/ml.

Amino acid sequencing

The protein was amino end cleared to remove the S-tag using a Thrombin cleavage Kit (Novagen). The digestion reaction was 5 µl 10 x Thrombin cleavage buffer, 0.5 mg purified recombinant protein, 1 µl of 0.01 µg/ml Thrombin which was left at room temperature for 18 hours. The reaction mix was run on a 12% SDS-PAGE gel and transferred onto PVDF membrane (Amersham, Chalfont, UK). This was stained with Coomassie Brilliant Blue and the protein bands destained and excised. Direct amino acid sequencing gave amino acids 28-32 of SEQ ID NO: 3 which matched the amino end (Department of Biochemistry, University of Cambridge).

Human recombinant antibodies

These peptides and the purified recombinant proteins were used to pan the phage display library. The peptide and recombinant protein were used at 10 mg/ml on NunC immunotubes Bst-N1 fingerprints of the PCR-amplified ScFv inserts before panning showed a highly heterogeneous library. After panning against peptide 1, 7 fingerprints were identified of which four were represented by more than one clone (A, B, C, D). These were combined as a pool for a neutralisation assay (pool 1) (below). After panning against peptide 2, clone A was present as well as a new ScFv, E. A and E were combined to produce pool 2. Against the clone recombinant fragment ScFvs E, F and G were present as well as a further ScFv, H. ScFvs E, F, G and H were tested together as pool 3.

Neutralisation assays

Chang cells (50 ml of 10^6 cells/ml) in maintenance media were grown overnight at 37 °C with 5% CO_2. Chang cells (1 ml of $1x10^6$ cells/ml maintenance media) were grown overnight at 37 °C with 5% CO_2 in plastic bijoux containing a thin glass circle on which the cells can grow. For recombinant protein or peptide assay (0.1 µl/ml), 100 µl of each sample was incubated with shaking for 1 hour with the cells at 37 °C. For the phage and sera assays, 100 µl of each sample (1:10 rabbit sera or dialysed phage pools 1-3) were incubated with 100 µl elementary bodies (EB) for 1 hour at 37 °C, shaking. After this first incubation, the 100 µl EB or 200 ml of the phage or rabbit sera/EB mix was added to the Chang cells. This was incubated with shaking for 1 hour at 37 °C. The supernatant was removed from every sample and replaced by 1 ml of fresh maintenance media. This was incubated at 37 °C with 5% CO_2 for 72 hours.

For both assays, the inclusion bodies were fixed and stained the following way; the cells were washed twice with PBS, then fixed with 100% methylated spirits for 10 minutes and washed twice again with PBS. The glass circles were incubated for 30 minutes with 10 µl of mouse *C.pneumoniae* inclusion bodies monoclonals (Mab) then washed 3 times with PBS and incubated for 30 minutes with 100 µl of fluorescein conjugated anti-mouse IgG. The inclusion bodies were then observed by fluorescence microscopy and three 200X fields counted. EB only samples were used as a positive control for chlamydial infection and dialysed phage supernatant without EB as a negative control.

Results

See Table 4 (Table of Neutralisation Assays).

Conclusion

Pre-incubation with the rabbit antiserum against peptide 2 and peptide 2 itself reduced the infectivity due to *C.pneumoniae*. Incubation with peptide 1 produced a similar reduction . The pools of phages were also active.

Overall this demonstrated the immunogenicity of the antigen, the potential therapeutic effect of peptides representing its key epitopes and both rabbit hyperimmune antiserum and ScFvs against these epitopes.

Table 1

Apparent Molecular Weight (kDa)	Group B (N=18)		Group C (N=18)		Group D (N=27)		Group E (N=21)	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
180	1	2		2	1	6		1
130		2			1	4		
120	1	5		1	1	5		1
98		5		1	2	5		2
90		2				2		
67		2	5	1			1	1
60/62*	8	5	5		13	7	2	2
51	7	11	9	10	2	3	1	2
47	1	1	1		0	0	0	0
40	0	0	0	3	0	0	0	1
30		4	0	3		2		2

* runs as a doublet within 1 mm of each other

Table 2

		Value for [a]				
Well No.	Epitope SEQ ID NO	Group 1 (n = 3)	Group 2 (n = 6)	Group 3 (n = 2)	Group 4 (n = 3)	Group 5 (n = 8)
3	9	0.538±0.205	1.028±0.423	0.425±0.036	0.416±0.184	0.499±0.191
4		0.599±0.252	1.487±0.462	0.502±0.036	0.407±0.107	0.438±0.162
13	10	0.462±0.203	1.103±0.229	0.473±0.026	0.421±0.162	0.427±0.188
31	11	0.491±0.192	1.103±0.310	0.440±0.004	0.407±0.105	0.310±0.129
41	12	0.547±0.235	1.169±0.256	0.474±0.024	0.393±0.08	0.376±0.158
43	13	0.598±0.258	1.223±0.323	0.558±0.015	0.423±0.119	0.406±0.181
55	4	0.547±0.235	1.265±0.334	0.475±0.02	0.373±0.076	0.381±0.042
58	5	0.611±0.019	1.025±0.06	0.611±0.019	1.127±0.253	0.800±1.232
59	6	0.494±0.166	1.096±0.267	0.547±0.009	0.546±0.200	0.702±0.144
60	7	0.489±0.129	1.048±0.270	0.483±0.064	0.388±0.008	0.449±0.140
61		0.530±0.236	1.051±0.262	0.59±0.089	0.446±0.09	0.784±0.257
76	8	0.485±0.158	1.174±0.255	0.654±0.068	0.564±0.223	0.666±0.266
79	14	0.510±0.235	1.21±0.273	0.418±0.003	0.423±0.127	0.388±0.153

[a] Optical density ± Standard deviation

Table 3

	[a] Pre Serum	Post Serum
Peptide 1	0.055 ± 0.01	0.591 ± 0.06
Peptide 2	0.056 ± 0.01	0.507 ± 0.04

[a] optical density ± standard derivation

Table 4 - Table of Neutralisation Assays

	Number of Elementary Bodies in Three 200x Fields
Cell control (dialysed phage supernatant)	0
Cell control (elementary bodies)	30
Rabbit anti-serum	
Versus peptide 1	30
Versus peptide 2	19
Pre-incubation	
Peptide 1	13
Peptide 2	0
Recombinant protein	12
Phage Pools	
Pool 1	18
Pool 2	N/D
Pool 3	21